# PATENT COOPERATION TREATY

# PCT

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# INTERNATIONAL PRELIMINARY EXAMINATION REPORT PCT

(PCT Article 36 and Rule 70)

23 MAR 2005

| Applicant's or agent's file reference<br>Case21414 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/EP 03/10294 | International filing date *(day/month/year)*<br>16.09.2003 | Priority date *(day/month/year)*<br>27.09.2002 |
| International Patent Classification (IPC) or both national classification and IPC<br>C12P23/00 | | |
| Applicant<br>DSM IP ASSETS B.V. et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 09.03.2004 | 21.01.2005 |
| Name and mailing address of the International preliminary examining authority:<br>European Patent Office - P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040 Tx: 31 651 epo nl<br>Fax: +31 70 340 - 3016 | Authorized Officer<br>Schneider, P<br>Telephone No. +31 70 340-4523 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/10294

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

1-9 as originally filed

**Claims, Numbers**

1-7 received on 05.10.2004 with letter of 05.10.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-7 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-7 |
| Industrial applicability (IA) | Yes: | Claims | 1-7 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: US-B1-6 291 204 (PASAMONTES LUIS ET AL) 18 September 2001 (2001-09-18)
D2: US-B1-6 365 386 (HOSHINO TATSUO ET AL) 2 April 2002 (2002-04-02)
D3: EP-A-1 111 067 (HOFFMANN LA ROCHE) 27 June 2001 (2001-06-27)
D4: MISAWA NORIHIKO ET AL.; BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 209, no. 3, 1995, pages 867-876

**1 Amendments** (Art. 28 PCT)

A new set of claims has been filed with a letter dated 05.10.2004, received 05.10.2004. The subject matter of new claim 6 relates to a process carried out at a pH in the range of 3 to 7. This specific pH range is not disclosed in the application as filed and therefore constitutes unallowable subject matter under Art. 28(2) PCT.

**2 Novelty** (Art. 33(2) PCT

No prior art document can be seen that discloses a ß-carotene accumulating microorganism of the genus Xanthophyllomyces (Phaffia) that additionally expresses a ß-carotene ketolase gene from *Agrobacterium, Alcaligenes, Paracoccus* or *Haematococcus*. The subject matter of claims 1 to 7 is therefore new under Art. 33(2) PCT.

**3 Inventive Step** (Art. 33(3) PCT EPC)

**3.1** D2 is the closest prior art and discloses the carotenogenic yeast strain *P. rhodozyma* ATCC96815 (e.g. col. 8, 3rd par.) which is blocked for the production astaxanthin and which

accumulates e.g. echinenone and canthaxanthin (col. 4 2nd par.) and which was stated to be used by the present application (see page 8, example 2). D2 also discloses the asthaxanthin biosynthetic pathway with all intermediates (i.a. echinenone and canthaxanthin produced by crtW, FIG.1)
From D2 the present application differs in that a ß-carotene ketolase gene (crtW) from *Agrobacterium, Alcaligenes, Paracoccus* or *Haematococcus* is expressed in said yeast strain.
The technical effect that is associated with said difference in the enhanced production echinenone and canthaxanthin. The simplification of their purification is already inherently comprised by D2 due to the absence of astaxanthin.
The problem to be solved is therefore the provision of an enhanced production process for echinenone and canthaxanthin.

**3.2** Document D1 relates to the improved fermentative production of carotenoids, i.a. echinenone and canthaxanthin (col. 1, 2nd and 4th par.), therefore the skilled person trying to solve the technical problem posed would clearly combine D1 and D2.
D1 discloses the ß-carotene ketolase genes crtW from *Agrobacterium, Alcaligenes PC-1* and *Haematococcus* to be expressed in *E.coli* for the production of canthaxanthin via echinenone (col.1). It also mentions the carotenogenic fungus Phaffia (written as "Pfaffia" which is an obvious error).
By combining D1 and D2 the skilled person would clearly introduce the crtW genes from D1 (or from any other microorganism) into the Phaffia strain from D2 and would arrive at the subject matter of the present application with a reasonable expectation of success without exercising inventive skills. As a consequence, the subject matter of claims 1 to 7 is not inventive under Art. 33(3) PCT.

**4** Apart from the above-mentioned the present claims fulfill the requirement of industrial applicability (Art. 33(4) PCT).

**5 Clarity** (Art. 6 PCT)

The term "substantially homologous" used in claim 5 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject matter of said claim unclear (Art. 6 PCT).

PCT/EP03/10294
DSM IP ASSETS B.V.

**Claims:**

1. A process for producing canthaxanthin and echinenone which comprises cultivating a recombinant microorganism which is expressing a β-carotene ketolase gene and belonging to the genus *Xanthophyllomyces* (*Phaffia*) and which accumulates β-carotene in an aqueous nutrient medium under aerobic conditions, and isolating the resulted carotenoids from the cells of said recombinant microorganism or from the cultured broth, wherein the β-carotene ketolase gene is originated from a microorganism which is selected from the group consisting of microorganisms of the genera *Agrobacterium*, *Alcaligenes*, *Paracoccus* and *Haematococcus* having the β-carotene ketolase gene.

2. The process according to claim 1, wherein the recombinant microorganism is derived from *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) ATCC96815, or a mutant thereof.

3. The process according to claim 1, wherein the β-carotene ketolase gene is originated from a microorganism which is selected from the group consisting of *Agrobacterium aurantiacum*, *Alcaligenes* PC-1, *Paracoccus marcusii* MH1, a gram-negative bacteria E-396 (FERM BP-4283), and *Haematococcus pluvialis*, having the β-carotene ketolase gene.

4. The process according to claim 1, wherein the β-carotene ketolase gene is originated from *Alcaligenes* PC-1 or the DNA sequence of the β-carotene ketolase gene is substantially homologous thereto.

5. The process according to claim 1, wherein the β-carotene ketolase gene is expressed in the recombinant microorganism using the control sequences.

6. The process according to claim 1, wherein the cultivation is carried out at a pH in the range of from 3 to 7 and at a temperature in the range of from 15 to 26°C for 24 to 500 hours.

7. The process according to claim 7, wherein the cultivation is carried out at a pH in the range of from 5 to 7 and at a temperature in the range of from 18 to 22°C for 48 to 350 hours.